The Impact of Iron Metabolism in the Pathogenesis of Multiple Sclerosis

Multipl Skleroz Patogenezinde Demir Metabolizmasının Etkisi

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ABSTRACT Objective: Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) leading to oligodendrocyte destruction, demyelination, remyelination, astrocytic scar formation, and neurodegeneration, all being associated with inflammation. Iron may contribute to the pathogenesis and progression of MS due to its accumulation in the human brain with age. It is unclear whether iron metabolism plays a role in the pathogenesis of MS. In this study, our aim was to investigate the possible role of dysfunctional iron metabolism in the pathogenesis of MS. **Material and Methods:** The study consisted of 32 patients with MS, and 30 age-matched healthy controls. Seven patients had relapsing remitting active (RRMS-A), 7 had relapsing-remitting stable (RRMS-S), 10 had secondary progressive disease (SPMS) and 8 had progressive relapsing (PRMS) disease. We analyzed hemoglobin, iron, transferrin and soluble transferrin receptor (sTfR) levels in MS patients and compared with controls. **Results:** There was no significant difference in terms of age between patients with MS (mean age, 36.85±7.23 years, n=32) and control group (mean age, 34.26±6.85 years; n=30). The sTfR levels were significantly higher in MS patients compared to the control group ($p<0.05$). **Conclusion:** The increased serum sTfR levels in MS patients may reflect an increased iron turnover, due to inflammatory and oxidative stres. Iron chelating therapies for MS patients can therefore, based on our current knowledge, not be recommended. Nevertheless, blocking harmful downstream effects of iron liberation, such as oxidation of lipids and DNA, might be beneficial for MS patients.

Key Words: Multiple sclerosis; iron metabolism disorders; receptors, transferrin

ÖZET Amaç: Multipl skleroz (MS) oligodentrositik destrüksiyon, demiyelinizasyon, remiyelinizasyon, astrositik skar oluşumu ve inflamasyon ile ilişkili nörodejenerasyona neden olan kronik bir santral sinir sistem (SSS) hastalığıdır. İnsan beyninde yaş ile birlikte birikim yapması nedeniyle demirin MS patogenezi ve progresyonunu tetikleyebileceği düşünülmektedir. MS patogenezinde demir metabolizmasının nasıl rol oynadığı net değildir. Biz bu çalışmada, MS patogenezinde olası demir metabolizması bozukluğunu değerlendirmeyi amaçladık. **Gereç ve Yöntemler:** Bu çalışma 32 MS hastası ve 30 sağlıklı kontrol bireyi içermekte olup, 7 hasta relapsing remitting aktif (RRMS-A), 7 hasta relapsing remitting stabil (RRMS-S), 10 hasta sekonder progresif (SPMS) ve 8 hasta progresif relapsing (PRMS) formundaydı. Biz MS hastaları ile kontrol grubu arasında hemoglobin, demir, transferin ve soluble transferin reseptör (sTfR) değerlerini analiz ettik. **Bulgular:** MS hastaları (ortalama yaş, 36,85±7,23 yıl, n=32) ve kontrol grubu (ortalama yaş, 34,26±6,85 yıl; n=30) arasında yaş açısından farklılık bulunmuyordu. sTfR değerleri MS hastalarında kontrol grubu ile karşılaştırıldığında anlamlı olarak yüksek bulundu ($p<0,05$). **Sonuç:** MS hastalarında artmış serum sTfR değerleri, inflamatuar ve oksidatif strese bağlı artmış demir dağılımını yansıtabilir. Bu nedenle, bilgilerimize göre, MS hastaları için demir bağlayıcı terapiler önerilemez. Bununla birlikte lipid ve DNA oksidasyonu gibi, demir salınımının zararlı ters etkilerinin bloke edilmesi MS hastaları için yararlı olabilir.

Anahtar Kelimeler: Multipl skleroz; demir metabolizması hastalıkları; reseptörler, transferrin

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Multiple sclerosis (MS) is a complex, genetically-predisposed immune disease in which self-reactive T cells and monocytes mediate inflammation of the central nervous system (CNS) white matter and demyelination of axons.[1]

Iron is vital for normal neuronal metabolism; however, its excessive amounts may be harmful.[2,3] Thus, the transport of iron across the blood-brain barrier (BBB) must be regulated. The transportation of iron across the BBB is mediated via several pathways. The transferrin/transferrin receptor may be the major route of iron transport across the luminal membrane of the capillary endothelium. The uptake of transferrin-bound iron is mediated by transferrin-receptor-mediated endocytosis. In addition to the transferrin receptor (TfR), several iron transport proteins contribute to iron transportation across the BBB. Once iron is transported across the BBB, it binds to the transferrin secreted by the oligodendrocytes (OGs) and choroid plexus epithelial cells. Within the brain, transferrin-iron (Tf-Fe) is taken up by neurons via a transferrin-receptor-mediated process.[4,5] A cell, which requires iron, expresses the TfR on its surface. In human plasma, a specific part of this receptor exists and is referred to as soluble transferrin receptor (sTfR), which is not affected by infection, inflammation, or any events increasing acute phase reactants. High iron concentrations in the brains of patients and dysregulation of iron may play a role in some neurodegenerative diseases, such as Alzheimer, Parkinson, and Huntington diseases, and Hallervorden-Spatz syndrome.[6]

Although there is great interest regarding the significance and impact of iron in the pathophysiology of MS, the contribution of disrupted iron metabolism in the pathogenesis has not been completely clarified. Iron accumulation in the MS plaques has been demonstrated, and high concentrations of iron have been determined in the OGs, myelins, reactive myelins and macrophages in the MS lesions. Iron is a necessary element for the maturation of OGs and gains greater importance in the presence of demyelination, since there is OGs damage or dysfunction in the pathogenesis of MS.[7,8]

The present study was aimed to determine the levels of serum iron, transferrin, and sTFR in patients with MS and to identify the possible relationship between levels of serum iron, transferrin, and sTFR and severity of MS with disease status.

MATERIAL AND METHODS

In the present study, 32 patients with MS were included and followed in Department of Neurology. The patients were diagnosed as MS by using McDonald criteria, and 14 of 32 patients fulfilled the criteria for Relapsing Remitting Multiple Sclerosis (RRMS), 10 patients were diagnosed with Secondary Progressive Multiple Sclerosis (SPMS), and 8 patients were diagnosed with Progressive Relapsing Multiple Sclerosis (PRMS).

We also obtained demographic and clinical data, such as gender, age, disease duration, and treatment given during the period of the study. The latter included glucocorticoids, immunomodulatory treatments (i.e., interferon-gamma), immunosuppressive drugs (azathioprine or mitoxantrone) or other treatments following immunomodulatory treatment. Patients with RRMS were classified as stable RRMS if they experienced no relapse within the past 9 months, and those with RRMS classified as active RRMS if they had symptoms at the time of the study, or had a relapse and one or more Gd-enhancing lesions within the last 6 months.

Secondary progressive MS was defined as having no relapse for at least 6 years and no radiological evidence of new lesion formation (no Gd-enhancing lesions and stable T2 lesion load as compared to a previous magnetic resonance imaging (MRI) ≥1 years).

The control group consisted of 30 gender and age-matched healthy subjects. The Expanded Disability Status Scale (EDSS) scores of the patients with MS were determined. Hematological tests including a hemogram, routine biochemical examinations, and thyroid function tests were performed. Evoked potentials including Visual Evoked Potentials (VEPs), Brainstem Auditory Evoked Potentials (BAEPs), and Somatosensory Evoked Potentials

(SSEPs) were assessed. All patients underwent contrast MRI, and the MRI of the patients were evaluated according to Mc Donald's criteria.[9]

The study received approval from the local ethics committees and adhered to the ethical guidelines of the most recent Helsinki Declaration (Edinburgh, 2000). Informed consent was obtained from all patients.

BLOOD SAMPLE COLLECTION

Ten cc of blood was obtained by venipuncture from each patient and centrifuged for 10 minutes at 3000 rpm in a tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The plasma samples were numbered and stored at -70°C. After completing the collection of the plasma samples of the study population, the levels of sTfR were measured by using a Biovender kit in a Triturus Enzyme Linked Immunosorbent Assay (ELISA) device in, Department of Biochemistry.

HEMATOLOGICAL PARAMETERS

The hematologic measurements included determination of hemoglobin, iron, transferrin and sTfR levels, Erythrocyte Sedimentation Rate (ESR), and C Reactive Protein (CRP).

STATISTICAL ANALYSIS

One-way analysis of variance (one-way ANOVA) was used to test the differences between the groups. The Tukey's test was carried out for variances which were homogeneous; the Tamhane test was carried out for variances which were not homogeneous in the post-hoc analysis. Pearson's correlation was performed to evaluate the relationship between the variables. The significance level was accepted as $p<0.05$.

RESULTS

The demographic and clinical features were presented in Table 1. The levels of serum iron, transferrin, hemoglobin, and sTfR in individual subgroups of patients with MS were presented in Table 2.

There was no significant difference in terms of age between patients with MS (mean age, 36.85±7.23 years, n=32) and control group (mean age, 34.26±6.85 years; n=30) (Table 1). The levels of serum iron, transferrin, and hemoglobin were within the normal limits in all patients with MS and there was no significant difference compared to the control group (Table 2).

TABLE 1: Characteristics of the participants.

	Patient group	Control group
Age (mean±SD;years)	36.85±7.23	34.26±6.85
Sex		
Female	20	16
Male	12	7
Disease duration (mean±SD; years)	4.2±3.24	
Disease course and activity		
RR-MS-A	n=7	(23%)
RR-MS-S	n=7	(23%)
RR-MS-T	n=14	(46%)
SP-MS	n=8	(31%)
PR-MS	n=10	(23%)
EDSS level (mean±SD; years)	4.53±1.84	

RRMS-A: Relapsing Remitting Multiple Sclerosis Active; RRMS-S: Relapsing-Remitting Multiple Sclerosis Stable; RRMS-T: Relapsing-Remitting Multiple Sclerosis Total; SPMS: Secondary Progressiv Multiple Sclerosis; PRMS: Progressive Relapsing Multiple Sclerosis; EDSS: The Expanded Disability Status Scale.

The levels of sTfR were significantly higher in patients with active RRMS, stable RRMS, SPMS, and PRMS compared to the control group ($p<0.005$). The levels of sTfR were higher in patients with active RRMS than patients with stable RRMS, SPMS, and PRMS ($p<0.005$). No significant correlation was found between EDSS scores and the levels of sTfR (Table 2).

MRI of the brain detected MS plaques in 20 subjects, most of which were periventricular in location. MRI of the cervical spine detected MS plaques in four subjects (eight patients had MS plaques in both brain MRI and cervical spine MRI).

Patients with positive brain and cervical spine MRI showed no significant correlation with the level of sTfR ($p>0.005$).

DISCUSSION

In recent years, data indicating iron regulation dysfunction in the pathogenesis of MS has increased.[10] Van Rensburg et al. found that serum iron concentrations were significantly lower than those in

TABLE 2: Some biochemical parameters of the MS patients.

Patient (n)	Iron (µmol/L)	Transferrin (g/L)	sTfR (mg/L)	Hemoglobin (g/dL)
RR-MS-A (7)	32.6±4.5	2.79±0.16	9.7±0.39*	12.7±0.85
RR-MS-S (7)	29.9±3.9	2.47±0.24	8.2±0.68*	12.4±0.73
RR-MS-T (14)	31.2±4.8	2.51±0.23	8.7±0.56*	12.5±0.79
SP-MS (8)	29.3±4.7	2.92±0.37	8.6±0.43*	13.1±0.93
PR-MS(10)	28.6±3.9	2.85±0.39	8.4±0.71*	12.4±0.76
Control (30)	28.6±4.5	2.69±0.36	5.8±0.89	12.5±0.85
EDSS				
0-5.5 (22)	28.1±3.6	2.79±0.28	8.8±0.73	12.8±0.83
6-10 (10)	28.4±4.1	2.72±0.39	8.6±0.69	12.5±0.72
Sex				
Male (20)	29.3±4.7	2.54±0.26	8.4±0.81	12.4±0.72
Female (12)	28.2±3.5	2.61±0.29	8.3±0.96	12.8±0.83

RRMS-A: Relapsing Remitting Multiple Sclerosis Active; RRMS-S: Relapsing-Remitting Multiple Sclerosis Stable; RRMS-T: Relapsing-Remitting Multiple Sclerosis Total; SPMS: Secondary Progressiv Multiple Sclerosis; PRMS: Progressive Relapsing Multiple Sclerosis; EDSS : The Expanded Disability Status Scale; sTfR: Soluble Tansferrin Receptor.
* $p<0.05$.

matched controls.[11] Sfagos et al. and Abo-Krysha and Rashed showed that there was no difference in the iron values between MS subgroups and control group.[12,13] In the present study no significant difference was found between the patients and controls as regards the level of iron.

Haemoglobin values as well as iron and transferrin levels were within normal limits in all patients. Transferrin has a key role in the metabolism of iron; since, it regulates iron flux between sites of absorption, storage, and utilization.[14] The transferrin concentration represents a measure for the specific iron transport capacity. The cells of the target organs regulate iron uptake via the expression of TfR, according to the individual iron requirements.[14,15] Zeman et al. found that serum transferrin values were lower (of borderline significance) in the patients with primary progressive MS compared to patients with the other forms of MS.[16] However, Sfagos et al. and Kotze et al. showed that there was no difference in the transferrin values between the subgroups of MS patients and the control group.[12,17] In the present study, also there was no difference in terms of levels of transferrin between the patients with MS and the control group. This finding may indicate that there is a balance among the factors which upregulate or downregulate the induction of transferrin expression.

Sfagos et al. and Abo-Krysha and Rashed have reported that the sTfR values were higher in patients with MS compared to controls.[12,13] Similarly, the results of the present study also demonstrated that sTfR values in patients with MS were significantly higher than controls. Levels of sTfR were significantly higher in active RRMS group compared to other forms of disease. Our results are consistent with the current literature.

TfR which is released by brain capillary endothelial cells may play a role in iron-related brain damage.[18] The endothelial cells in the BBB activate the release of adhesion molecules in order to increase the entry of T-lymphocytes into the CNS in MS. Both T-cell proliferation and the lymphokine release are depended on iron levels. The TfR release by lymphocytes is modulated by interleukin-2. Active endothelial cells and lymphocytes secrete TfR.[19-21] Consistent with the previous studies, a significant increase in sTfR values was found in patients with MS in the present study.[12,13] Surprisingly, it was demonstrated that sTfR values were also higher in patients with stable RRMS compared to the controls.

Haacke et al. showed that iron level is higher than normal in white matter, as well as in basal ganglia and thalamus.[22] This iron increment may underlie the chronic failure of cerebrospinal fluid

venous drainage during the disease course and in this way causes the occurrence of disease symptoms.[23] Hametner et al. found an age-related increase of iron in the white matter in controls as well as in patients with short disease duration. In chronic MS, however, there was a significant decrease of iron in the normal-appearing white matter corresponding with disease duration, when corrected for age.[24]

These results may point out the importance of iron in the treatment of MS. Iron treatment may be effective in the course of disease by regulating the neuronal iron metabolism. Therefore, for a better understanding of the changes detected in the metabolism of iron in the pathogenesis of MS, randomized, double-blind, multicentre, quantitative MRI techniques, and body fluids of iron, iron-related proteins examined studies are needed.

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